



TITLE:

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CITATION:

IWATA, Ryutaro ...[et al.]. <Original>Studies on the Autecology of *Lyctus brunneus* (STEPHENS) (Coleoptera, Lyctidae) : VII. Chemical Investigations of the Nutrient Composition of Foods. Wood research : bulletin of the Wood Research Institute Kyoto University 1986, 72: 45-51

ISSUE DATE:

1986-02-28

URL:

<http://hdl.handle.net/2433/53314>

RIGHT:

Studies on the Autecology of *Lyctus brunneus* (STEPHENS) (Coleoptera, Lyctidae)

VII. Chemical Investigations of the Nutrient Composition of Foods

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(Accepted November 16, 1985)

Abstract—Contents of amino acid and starch, as the two major indispensable nutrients for a wood-destroying beetle, *Lyctus brunneus* (STEPHENS), were compared between the foods (artificial diet; oak sapwood) and the feces. Although the degrees of utilization by this insect varied considerably among amino acids, the acidic amino acids and those with hydroxyl group were rather preferentially utilized. Starch in the samples was analyzed to obtain the ratio of amylose to amylopectin. The latter that was contained in greater amounts seemed to be more efficiently utilized by the insect. The value of the ratio of consumed quantity of starch to that of amino acids in the oak sapwood was higher than that in the artificial diet, indicating a higher requirement of energy in wood feeding than in diet feeding.

1. Introduction

The information concerning the nutritional aspects of the powder-post beetle, *Lyctus brunneus* (STEPHENS), is one of the most fundamental requirements for the control of this species that is causing damage on hardwood sapwood. As early as in 1893 and 1903, É. MER^{1,2)} suggested that starch might be the most vital ingredient in wood for *Lyctus*. This has been well established by a number of succeeding works³⁻⁸⁾. The second most vital nutrient for *Lyctus* is supposed to be proteins and/or amino acids⁶⁻¹¹⁾, with methionine and tryptophan being pointed out to be the most important⁸⁾. In addition, it has been briefly noted that a successful development of *Lyctus* can take place on a diet in which the carbohydrate: protein ratio is 15:1, whereas the normal ratio in wood is 3.3:1¹²⁾ although the type of carbohydrate has not been specified.

Previously, the authors' attention has been focused on the preparation of the

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artificial diet adequate for mass culture of this species^{13,14)}. During the course of these experiments, starch content in the diet was found to be much more important than protein content from quantitative viewpoint. Furthermore, these results^{13,14)}, indicate that the ratio of starch to protein is also an important factor for the development of the species.

In order to extend the previous studies, the present investigation was conducted to compare the amino acid and starch compositions of the wood and of the newly established standard artificial diet¹⁴⁾ with those of two kinds of feces of *Lyctus brunneus*.

2. Materials and Methods

2.1 Materials

The insects and materials subjected to the chemical analyses are as follows, with their code names headed:

D: Intact standard artificial diet¹⁴⁾ composed of air-dried 26% lauan (*Shorea* spp.) wood sawdust, 50% soluble starch (Nakarai Chemicals, Ltd.), and 24% brewer's yeast powder ("Ebios", Asahi Breweries, Ltd.). These values correspond to 27.4% lauan wood sawdust, 47.7% soluble, starch, and 24.9% brewer's yeast powder in an absolutely dried state.

DF: Frass (feces) of the insects taken out from the culture with the above artificial diet (D).

BA: Whole adult beetles (504 individuals) of *L. brunneus* supplied from a mass culture system¹⁵⁾ with the diet (D).

Q: Intact wood particles remaining in the heavily infested sapwood of an oak species, *Quercus serrata*, which was used for insect culture. Oak was cut about 5 years before and the wood was stored at 5°C before exposure to insect attack.

QF: Frass (feces) of the insects taken out from the culture with the oak wood specified in Q.

2.2 Amino Acid Analyses

All samples were extracted with organic solvent prior to amino acid analyses. About 1 g of BA was defatted with acetone at -10°C for 72 hr. Q was smashed and passed through a coarse sieve, while D was originally supplied in a powder state. Since DF and QF were obtained in a very fine powder state, these materials were directly passed through a No. 100 mesh sieve. D, Q, DF and QF were then extracted with ethyl alcohol-benzene (1 : 2, v/v) for 6-8 hr. Each extractive-free sample was divided into 2 parts: one was used for the general amino acid analysis and the other for tryptophan analysis.

The conditions of acid hydrolysis in the general amino acid analysis were

essentially identical with those of IJIMA *et al.*⁸⁾. Briefly, for general amino acid analysis, each sample was hydrolyzed with 6N hydrochloric acid for 24 hr at 105°C. The hydrolyzed solution was passed through Amberlite CG 120 column (NH₄⁺ form), which was then exhaustively washed with distilled water to remove components other than amino acids. Amino acids were then recovered by elution with 2N aqueous ammonia solution.

For tryptophan analysis, each sample was hydrolyzed with saturated barium hydroxide solution for 48 hr at 105°C under nitrogen¹⁶⁾. The hydrolyzed solution was neutralized with sulfuric acid, and the precipitated barium sulfate was removed by filtration. Tryptophan was recovered from this neutralized solution by passing it through Amberlite CG 120 column as described above. The amino acid content and composition were analyzed with Hitachi 835 Amino Acid Analyzer after the total amino acid content was adjusted to be in the range of 1–5 μ M/ml.

2.3 Starch Analyses

The material Q was slightly ball-milled (10 min), as well as D, to liberate amyloplasts from the parenchyma cells. DF and QF were passed through No. 100 mesh sieve to remove intact portions.

Starch was extracted from each powdered sample with perchloric acid in the same way as described in the standardized method of AOAC¹⁷⁾ except in that phosphowolframic acid was used as deproteination reagent instead of uranyl acetate. The amount of the solubilized starch was determined by the method of HUMPHREYS and KELLY¹⁸⁾ based on the color reaction with iodine using potato starch (Nakarai Chemicals, Ltd.) as the standard. The weight ratios of amylose to amylopectin in the starch were also determined by the iodine colorimetric method¹⁹⁾ using amylose and amylopectin (Sigma Chemical Company) as the standards.

3. Results and Discussion

The amino acid compositions of the foods (D; Q) and feces (DF; QF) together with the whole adult beetles (BA) of *L. brunneus* are summarized in Table 1. The results showed that acidic amino acids, such as aspartic acid and glutamic acid, and amino acids with hydroxyl group, such as serine and threonine in the artificial diet were highly utilized. When the results of D-DF and Q-QF were compared, utilization of amino acids were found to vary considerably and no importance of methionine and tryptophan was noted in contrast to the results of IJIMA *et al.*⁸⁾. Since the lauan wood sawdust added to the artificial diet seems to contain relatively vrey small amount of protein as shown in the oak wood (Q), yeast powder in the diet must contribute the major source of protein in the artificial diet.

Increases in the relative amounts of some amino acids such as lysine, glycine,

Table 1. Amino acid compositions (μ moles/g)

Amino acid	Sample				
	D	DF	Q	QF	BA
Lys	12.1	22.5	1.9	1.3	172.7
His	trace	0.7	0.2	0.1	19.9
Try	3.1	1.6	0.1	trace	7.5
Arg	15.4	10.6	3.6	4.0	74.5
Asp	63.4	2.2	0.2	0.1	4.4
Thr	23.9	0.8	0.2	0.1	1.0
Ser	35.1	2.1	0.3	0.2	4.9
Glu	78.6	5.6	1.5	0.5	14.9
Pro	33.0	8.3	trace	0.2	16.6
Gly	13.4	25.8	1.0	0.7	97.9
Ala	57.5	36.2	0.8	0.4	86.5
1/2 Cys	2.7	1.9	0.5	0.2	7.8
Val	44.0	23.1	0.1	0.2	85.3
Met	trace	6.0	0.3	0.1	15.1
Ileu	30.1	14.9	1.6	0.6	123.1
Leu	45.3	34.0	4.8	2.5	211.8
Tyr	9.3	10.4	0.9	0.6	101.6
Phe	16.6	19.1	2.7	1.6	160.7
Total amino acid contents (%) ^{a)}	6.13	2.84	0.30	0.20	16.53

a) The value is expressed as a percentage of the dry weight of each sample.

tyrosine and phenylalanine in D-DF were observed after the digestion by *Lyctus*. This might be due to the metabolic actions by the insect and/or the symbiotic microorganisms present in the gut of the insect²⁰⁾. The newly presented data of the amino acid composition of the whole bodies of adult beetles (BA) will be expected to be useful in making an adequate holidic diet for biochemical studies.

In the next place, we pay attention to the starchy polysaccharide content and composition in the foods and feces. Previously starch contents in sapwoods of various wood species have already been reported, the values being widely varied

Table 2. Starch contents in the foods and feces^{a)}

Sample	D	DF	Q	QF
Starch content (%)	47.7	14.2	3.22 \pm 1.17	0.91 \pm 0.11
Ratio of amylose to amylopectin	2 : 98	14 : 86	14 : 86	17 : 83
Amylose content (%)	1.0	2.0	0.43 \pm 0.14	0.15 \pm 0.01
Amylopectin content (%)	46.7	12.2	2.79 \pm 1.03	0.76 \pm 0.10

a) Based on the dry weight of each sample.

from 0.2% to 8.7%⁸⁾, and a comparison has been made between the values of wood and *Lyctus* frass. However, the comparison of starchy polysaccharide analyses of diet and feces from diet culture has not been carried out yet. Table 2 summarizes the results of the starch contents and the ratios of amylose to amylopectin in the foods and feces. The starch content of oak wood (Q) (3.22%) was in good agreement with the minimum value (3%) of those observed in the sapwoods which could be infested by *Lyctus*⁸⁾. A small amount of starch was found to remain in the feces, indicating that this insect could not always utilize starch in the diet completely.

The ratio of amylose and amylopectin, as well as their amounts, are also shown in Table 2. A remarkably low amylose content in the artificial diet (D) may be ascribed to the fact that a partially hydrolyzed water-soluble starch was utilized as the starch component. The starch in oak wood (Q) also comprised amylopectin for the most part. The ratios of amylose to amylopectin in the feces were slightly higher than those in the intact foods. These results indicate the preferential utilization of amylopectin by *Lyctus*. This seems a kind of physiological adaptation of the insect to the food nutrition as an environment.

Changes of the nutrient contents in the foods by *Lyctus* digestion were calculated based on the results shown in Tables 1 and 2. The results are summarized in Table 3.

Table 3. Changes of the nutrient content in the foods by *Lyctus* digestion

		Diet digestion (Comparison of D and DF)		Wood digestion (Comparison of Q and QF)	
Composition of food (D or Q) (%)	Starch	47.7	(D)	3.22	(Q)
	(Amylose)	(1.0)		(0.43)	
	(Amylopectin)	(46.7)		(2.79)	
	Amino acids	6.0		0.30	
	The other	46.3		96.48	
Composition of frass (feces) ^{a)} (DF or QF) (%)	Starch	{14.2}	(DF)	{0.91}	(QF)
	(Amylose)	{8.0}		{0.89}	
	(Amylopectin)	{1.1}		{0.15}	
	(Amylopectin)	{6.9}		{0.74}	
	Amino acids	{3.5}		{0.20}	
	The other	{82.3}		{98.89}	
Difference between food and frass (%) (Quantity consumed (g) per 100 g of food)		Starch	39.7	2.33	
		Amino acids	4.0	0.10	
Utilization ratio (%) (Ratio of quantity consumed to the initial content in food)		Starch	83	72	
		Amino acids	67	33	
Ratio of consumed quantity of starch to that of amino acids		9.9 : 1		23.3 : 1	

a) Composition values of frass in parentheses { } were transformed so that the values of "the other" are fixed to those of foods.

More than 70% of starch originally present in the foods proved to be utilized (83% in the artificial diet and 72% in the oak wood). Although 67% of amino acids in the artificial diet was utilized, only 33% of amino acids in oak wood could be utilized. The ratios of the consumed starch and amino acids in the diet (D-DF) and wood (Q-QF) were 9.9:1 and 23.3:1, respectively. This low utilization ratio of amino acids, and therefore the high utilization ratio of starch in wood, would be reasonable since a higher amount of energy transformed from starch would be required in masticating a harder and nutritionally poor food such as wood. Furthermore, it should be noted that D and Q showed rather similar values of the ratios of starch to amino acids (8.0:1 for D and 10.7:1 for Q). These results confirmed the previous observation that the content of starch was more important than that of protein. The similarity in the ratios of starch to amino acids between the artificial diet (D) and the oak wood (Q) may indicate a suitable nutrient balance of the diet.

4. Conclusions

- (1) The degrees of amino acid utilization by *Lyctus brunneus* varied considerably among amino acids, with acidic amino acids and those with hydroxyl group being rather preferentially utilized.
- (2) No indispensable amino acids for the development of *L. brunneus* could be specified.
- (3) Amylopectin portion of starch seemed a better nutrient for *L. brunneus* than amylose, and the oak wood starch comprised the former for the most part.
- (4) Feeding wood seemed to require much energy in the form of carbohydrate (starch) in comparison with feeding the artificial diet.

Acknowledgement

The authors express their sincere gratitude to Prof. Tetsuo KOSHJIMA, Wood Research Institute, Kyoto University, for his kind suggestion and encouragement.

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